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"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

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SUMMARY

During the past quarter, we have continued our physiologic and histologic studies of safe stimulation of cortical neurons using our feline model of the sensorimotor cortex. Both 7-electrode and 16-electrode arrays were used and 3, 4 or 5 of the microelectrodes in the 7-electrode array or 11 microelectrodes in the 16-electrode array, were pulsed continuously for 7 hours or more. In the physiological evaluations, we have previously reported variable degrees of stimulation-induced depression of neuronal excitability (SIDNE) using both interleaved and simultaneous stimulation with multiple microelectrodes. These studies were described in detail in QPR's #3 and #7. In this report, we describe the correspondence between the physiologic and histologic (LM and TEM) changes during and following the intracortical microstimulation. These studies have confirmed the elevation of activation thresholds of the corticospinal neurons; however, histologic studies of the same animals failed to demonstrate the presence of any histologically-detectable neuronal or neural injury, over a wide range of electrical parameters; i.e., frequency (50-500 Hz), charge densities (200-800 μC/ph), (4.0 - 8.0 nC/ph), duration of stimulation (7, 14 or 21 hours), or whether the electrodes were pulsed simultaneously or in the interleaved mode.

METHODS

Fabrication of the microelectrode arrays. The shafts of the discrete iridium microelectrodes are made from iridium wire, 35 μm in diameter. One end of each shaft is etched electrolytically to a cone with an included angle of 10° and with a blunt hemispheric tip approximately 12 μm in diameter. After the tips have been shaped to the proper configuration, a Teflon-insulated wire lead is micro-welded near the upper end of the shaft. The shaft is then insulated with 4 thin coats of Epoxylite electrode varnish, and each layer of insulation is baked using a schedule recommended by the manufacturer. The insulation is removed from the tip of the shafts by an erbium laser. The surface area of the exposed tip is determined by measurement of the double-layer capacitance while the tip is immersed in phosphate-buffered saline solution, using fast (100 Hz) cyclic voltammetry. The surface areas of these electrodes was 500 ± 100 μm^2

or 2,000 ± 400 μm^2. The individual microelectrodes are then assembled into arrays of 7 or 16, which extend 1.1 to 1.2 mm from an epoxy matrix, which is 3 mm in diameter. In addition, the arrays contain 3 electrically inactive stabilizing pins, whose tracks also serve as fiducial marks for identifying the individual electrode during the subsequent histologic analysis (Figs. 1A and 1B). The microelectrodes are then "activated" (a layer of high-valence iridium oxide formed by anodic conversion) by potentiodynamic cycling between -0.8 and +0.7 volts with respect to a saturated calomel electrode, with the microelectrodes immersed in saturated sodium phosphate solution. The activation process is terminated when each microelectrode has a total charge capacity of 20 nC.

Cleaning procedure for microelectrodes. The completed arrays of 7 or 16 microelectrodes were placed in a 1 liter beaker and the tips protected by clamping an aneurysm clip on the cable. They were then sonicated in deionized water (10 M Ohm) for 30 seconds. This was followed by a 1-hour soak in 10% "7×" detergent and then rinsed 7 times by sonication in deionized water. Finally, the microelectrodes were soaked for 24 hours in deionized water to allow leaching of any residual impurities. In recent implants (animals # 189 to 198), we have added a 3-minute sonication of the arrays in 95% alcohol, followed by 3 minutes of sonication in deionized water, performed in the surgical suite within 45 minutes of the time of the implant.

Surgical Procedure. Aseptic technique is used during the surgical implantation of the microelectrode arrays. Young adult cats of either sex are anesthetized initially with Ketamine with transition to a mixture of nitrous oxide, oxygen and Halothane. The surgical procedure is carried out with the animal's head in a stereotaxic apparatus. The scalp and temporalis muscle are reflected and, using a Hall bone drill, a craniectomy is made over the left frontal cortex extending into the frontal sinus. The frontal air sinus is partly filled with bone cement.

Prior to implanting the intracortical microelectrode array, a monopolar recording electrode, and its accompanying reference electrode, are implanted in the cat's pyramidal (corticospinal) tract in order to record neuronal activity evoked by the

stimulating microelectrodes. The recording electrode is fabricated from 0.25 mm Teflon-insulated stainless steel wire. The exposed area at the tip of the stainless steel electrode wire is approximately 0.1 mm^2. In preparation for implantation, the wire is mounted in a sleeve-type cannula device, which is mounted in a stereotaxic assembly. A small burr hole is cut in the calvarium over the cerebellum, and a small incision is made in the dura. A stimulating macroelectrode (approximately 0.5 mm in diameter) is placed against the dura over the pre- or postcruciate gyrus. This macroelectrode can support a large stimulus current (1 to 2 mA, 150 μsec/ph, at 20 Hz) which excites many corticospinal neurons and thus produces a large compound action potential that can be used to guide the recording electrode into the pyramidal tract. When the tip of the electrode is in the pyramidal tract, the inner introducer is retracted, and the recording and reference electrodes are sealed to the skull with bone cement.

In preparation for implanting the microelectrode array into the sensorimotor cortex, the percutaneous connector is mounted to the skull with stainless steel screws and methacrylate bone cement. A small flap, slightly larger than the array's superstructure matrix, is made in the dura over the postcruciate cortex, and the array of microelectrodes is inserted into the cortex with the aid of an axial introducer mounted on the stereotaxic frame. Prior to animal IC-176, the array was inserted slowly, at approximately 0.25 mm/sec. In the later animals, the arrays were implanted at a velocity of approximately 1m/sec. In our cat model, we have found that it is best not to suture the dura over the array, but we do cover the array with a sheet of fascia resected from the temporalis muscle. The cortex and fascia are covered with Gelfoam and the skull defect is sealed with cranioplasty.

Stimulation Protocols. The test stimulation protocols were conducted at least 30 days after the implant surgery. During the stimulation, the cats were lightly anesthetized with Propofol, and the stimulation and recording was conducted through a percutaneous connector fixed to the skull. We have determined that the electrical excitability of the corticospinal neurons is not altered by light Propofol anesthesia, and the cats are much easier to manage when lightly anesthetized than when awake and

wearing the tethered backpack. In each array, 3 to 5 of the 7 microelectrodes in the 7-electrode array, or 11 electrodes in the two animals with 16-electrode arrays, were pulsed continuously for 7 hours. The microelectrodes were pulsed either simultaneously or sequentially (interleaved stimulation). The stimulus was charge-balanced, controlled current biphasic pulse pairs, 150 μs/phase (cathodic phase first). The activated iridium microelectrodes were biased at +400 mV with respect to the implanted Ag/AgCl reference electrode. The stimulation protocols are presented in Table 1.

Recording from the pyramidal tract. The neuronal activity evoked in the ipsilateral pyramidal (corticospinal) tract by each of the intracortical microelectrodes was recorded before and after the sessions of continuous stimulation. The response to 1048 to 4096 successive stimulus pulses were summated (averaged), in order to obtain an acceptable signal-to-noise ratio. When a response was present, it usually had the characteristics of having been generated by a single corticospinal neuron. This is due apparently to the sparseness of the corticospinal projection, and the fact that any corticospinal neurons that are excited from a particular microelectrode must pass very close to the pyramidal tract recording electrodes, in order for their action potentials to be detected. Such "unit-like" responses can be identified by their "all or none" character and by their discrete latency after the stimulus pulse, as described in QPR's #3 and #7.

Physiological assessment of electrode stability. To assess the stability of the interface between neural tissue and the stimulating microelectrodes, neural activities were recorded from the implanted electrodes on a weekly basis for periods up to 18 months (IC-175). An algorhythm based on cluster analysis and interspike interval analysis was developed to sort the extracellular potentials into single units. The extent and timing of electrode movement was determined and expressed quantitatively by a stability index (Liu, 1999).

TABLE 1

STIMULATION PARAMETERS

IC #	LM/ EM	POST STIM.	STIM. DURATION	SIMUL./ INTER.	Hz	µA/ph	nC/ph	PULSE DURATION	CHARGE DENSITY	ELECTRODES PULSED	SACRIFICED POST-IMPL.
169	LM	4 days	7 hrs.	Inter.	50	26.5	4.0	150 µs	800 µC/cm² *	1,2,5,6,7	47 days
170	LM	45 min.	4 days (7 hrs/day)	Simul.	50	26.5	4.0	150 µs	800 µC/cm² *	1,3,5,6,7	60 days
172	LM	45 min.	4 days (7 hrs/day)	Simul.	500	26.5	4.0	150 µs	200 µC/cm²	2,3,4,5,6	103 days
174	LM	48 hrs.	7 hrs.	Inter.	500	26.5	4.0	150 µs	200 µC/cm²	2,4,5	165 days
175	LM	48 hrs.	7 hrs.,	Simul.	500	53.0	8.0	150 µs	800 µC/cm²	1,2,3,4	18 months
176	EM	48 hrs.	(1) 7 hrs. (2) 7 hrs.	Simul. Simul.	50 500	26.5 26.5	4.0 4.0	150 µs 150 µs	200 µC/cm² 200 µC/cm²	2,3,4 5,6,7	129 days
190	EM	(Immed.)	7 hrs.	Simul.	500	26.5	4.0	150 µs	200 µC/cm²	3,4,6,7	160 days
192	LM	48 hrs.	7 hrs.	Simul.	50	26.5	4.0	150 µs	200 µC/cm²	1,3,4,5,6,7,8,9, 10,13,14	28 days
196	LM	Immed.	(1) 7 hrs. (2) 7 hrs. (3) 7 hrs.	Simul.	100 100 100	20.0 20.0 20.0	8.0 8.0 8.0	400 µs 400 µs 400 µs	400 µC/cm²	1,3,5,7,10,11, 12,13,16 (all 3 days) 2,4,8,14,15 (8 hrs. only)	28 days

*The geometric surface areas of the electrodes in IC-169 and IC-170 were 500 ± 100 µm². The surface areas of the electrodes in the other animals were 2,000 ± 400 µm².

Histologic Methods. Thirty minutes to 48 hours after the end of the stimulation regimen, the cats were deeply anesthetized with pentobarbital, heparinized, and one liter of 0.9% NaCl perfused through the ascending aorta using a peristaltic pump (Model #2520-55, Cole Palmer Instrument Co.) at 120 mm Hg. This prewash was followed by one liter of ½ strength Karnovsky's fixative (2.5% glutaraldehyde, 2% paraformaldehyde and 0.1 M sodium cacodylate buffer). This procedure was used both for light (LM) and transmission electron microscopy (TEM) preparation.

For LM preparation, the brain was left in fixative overnight and, the following day, the array of microelectrodes was removed from the cerebral cortex after careful dissection of the overlying connective tissue and dura. The block of tissue containing the array tracks was resected, embedded in paraffin, sectioned serially in the horizontal plane (perpendicular to the shafts of the stimulating microelectrode) at a thickness of 8 μm. Alternate sections were stained with cresyl violet (Nissl stain) and H&E. Occasional sections near the electrode tips were stained with an antibody to glial fibrillary acidic protein (GFAP).

The same perfusion procedure and fixative was used for preparation of tissue for TEM. Following perfusion, 1 × 1 × 2 mm blocks were cut from the cortex. In addition to the tissue around electrode tracks, control tissue was taken from the contralateral cortex. The tissue was embedded in Polybed 812 epoxy, and sectioned in the horizontal plane (perpendicular to the electrode shafts) at 1 μm intervals. In 2 animals designated for electron microscopy, the 1 μm sections were used to choose specific areas for ultrathin sections, which were stained with uranyl acetate and lead citrate.

RESULTS

Physiological results. Fig. 2 shows the effects of 7 hours of continuous stimulation, at a pulse amplitude of 26.5 μA (4 nC/ph), 150 μs/phase, and at a pulse rate of 50 Hz, on the threshold of 7 unitary-like responses recorded from the corticospinal tract of 2 cats, including IC-176. The histologic findings from this animal

are described below. In the figure, the abscissa is the electrical threshold of the unit-like pyramidal tract response before the start of the 7 hours of continuous stimulation, and the ordinate is the threshold immediately after the end of the 7 hours. The diagonal line represents the condition in which threshold before stimulation = threshold after stimulation (i.e., the condition in which the 7 hours of stimulation had no effect on the neural response threshold). The latency of the unit-like responses after the stimulus pulse was less than 2 ms, so we presume that they were activated directly by the intracortical microstimulation rather than transsynaptically.

In cat IC-176, after the 7 hours of stimulation at 50 Hz, a second group of 3 microelectrodes was pulsed simultaneously for 7 hours at a pulse amplitude of 26.5 μA and at 500 Hz (Fig. 2). The cat was then perfused for histologic analysis. In cat IC-190, 4 of the 7 microelectrodes were pulsed simultaneously for 7 hours, also at 4 nC/ph and at a pulse rate of 500 Hz. Fig. 3 shows the effect of this high-rate stimulation on one response from cat IC-176 and on three responses from cat IC-190. This high-rate stimulation always caused an increase in the threshold of the corticospinal neurons.

In cat IC-192, 11 of the 16 microelectrodes were pulsed simultaneously for 7 hours, at 50 Hz and at 26.6 μA (4 nC/phase). This regimen induced increases in the thresholds of some of the neuronal responses (Fig. 4).

Recording from stimulating microelectrodes. Recording of neuronal activity from the intracortical microelectrodes was carried out in most of the animals listed in Table 1. Animal IC-175 was followed for 18 months and the results are representative for this procedure. The array of 7 intracortical microelectrodes was implanted into the postcruciate gyrus on April 21, 1999. Recording of the action potentials from individual cortical neurons ("neural units") began approximately 1 month after implantation. During the next 3 months (1-4 months after implantation), neural units could be recorded from all 7 microelectrodes, but the recordings tended to be somewhat unstable, in that the action potentials from a particular neuron could be recorded during one session but not during a subsequent session a few days later. Conversely, the action potentials from different cortical neurons would appear in subsequent sessions.

We attribute this instability to slow migration of the tips of the electrodes through the tissue. After the 4th month, the recordings became more stable and the action potentials from particular neurons could be recorded for weeks at a time. We interpret this as evidence of reduced rate of migration of the array through the tissue. However, by the time the cat was sacrificed 18 months after implantation of the array, neural units could be recorded only from 4 of the 7 microelectrodes (electrodes #4, #5, #6, #7).

HISTOLOGICAL RESULTS

Mechanically-induced histologic effects. In the previous contract period we evaluated a number of characteristics of microelectrodes and insertion procedures which were potential contributors to mechanically-induced damage, including microhemorrhages (in acute experiments) and resolved cavitations (originally microhemorrhages) in chronic implants. The results of these studies were summarized in the final QPR, February 28, 1998, and in QPR #5 of the present contract. Microhemorrhages were significantly reduced by the use of a slimmer iridium shaft (35 μm), a blunter (12 μm) electrode tip and the use of a stereotaxically-mounted electrode inserter by which the arrays are inserted at a speed of approximately 1m/sec. While microhemorrhages have not been eliminated by these steps, they have been significantly reduced, both in frequency and the size of the cavitations. The most prominent histologic changes in both pulsed and unpulsed arrays have been vascular hypertrophy and hyperplasia, particularly between the electrode tracks of the multiple electrode arrays. However, these vascular changes tend to vary in density at various levels in the cortex. For example, Fig. 5 shows heavy hyperplasia amongst the tracks of a 7-electrode array at a depth of 200 μm beneath the pia (IC-175), whereas the same array showed sparse hyperplasia at a depth of 300 μm (Fig. 6). Another frequent observation is the flattening of neurons at the tips and adjacent to a portion of the perimeter of electrode tracks, probably due to lateral movement of the electrode (Figs. 7 & 8). If this occurred during implantation of the array, it is apparently irreversible over a period of several months; however, the neurons stain normally and are apparently functional (Fig. 7). Evidence of lateral movement was frequently seen at the tips or

along the shafts of both stimulating and stabilizing electrodes. This movement was invariably greater at the tips of stabilizing electrodes, presumably due to their greater length (2.5 mm). Such movement can be identified by a glial scar, enhanced in GFAP-stained paraffin sections (Fig. 8).

Electrically-induced effects of microstimulation. Table 1 presents the protocols for microstimulation of arrays of 7 or 16 microelectrodes chronically-implanted in the sensorimotor cortex of 9 cats. Seven of the nine animals were evaluated by light microscopy and two by transmission electron microscopy. There was no evidence of electrically-induced neuronal (or neural) injury by either LM or TEM evaluation in any of the animals listed in Table 1. These experiments included three animals (169, 174 and 190) in which the microelectrodes were pulsed continuously for 7 hours (one with simultaneous stimulation, two with interleaved stimulation) at a pulse amplitude of 26.5 μA, 4 nC/ph, 50 or 500 Hz and 150 μs pulse duration. Histologic changes adjacent to the tips of pulsed electrodes (Fig. 9) were essentially the same as those near unpulsed electrodes (Fig. 10). Animal IC-192 was implanted with a 16-electrode array, and 11 electrodes in each were pulsed simultaneously at 50 Hz, for 7 hrs/day. The pulse amplitude was 4 nC/ph. Histologic evaluation revealed no neuronal injury near or between the 4 pulsed sites.

Prolonged stimulation experiments included 2 animals that were stimulated at 4nC/phase for 7 hrs/day on 4 successive days at frequencies of 50 Hz (IC-170) or 500 Hz (IC-172). A third animal (IC-196), implanted with a 16-electrode array was stimulated for 7 hrs/day on 3 successive days at 8 nC/ph and at 100 Hz. In that animal, nine electrodes were pulsed on all 3 days, five electrodes were pulsed for 7 hours only on the first day and 2 microelectrodes were not pulsed on any of the 3 days. In all cases, the tissue surrounding the unpulsed electrodes was essentially indistinguishable from that adjacent to the unpulsed electrodes, i.e., no histologic changes could be attributed to electrical stimulation (Figs. 11 & 12).

Two animals (176 and 190) were evaluated by transmission electron microscopy to assess intracellular changes. In cat IC-176, three electrodes were pulsed for 7 hours

at a frequency of 50 Hz and at 4.0 nC/ph and the other 3 were pulsed at a frequency of 500 Hz. In cat IC-190, four microelectrodes were pulsed for 7 hours at a frequency of 500 Hz at 4.0 nC/ph and 3 were unpulsed. Fig. 13 is a micrograph of the tissue surrounding the track of an unpulsed electrode from IC-190, showing normal-appearing neurons, neuronal organelles and neuropil. Fig. 14 is a light-level micrograph of a semi-thin section through the track of pulsed electrode #5 from animal IC-190, about 90 μm above the tip site. The nearby neurons appear quite normal. Figs. 15 shows an electron micrograph of the tissue just above the tip site of a microelectrode from IC-176, pulsed continuously with 50 Hz, 26.5 μA (4.0 μC/ph) for seven hours. Several normal-appearing neurons are closely adjacent to the electrode tip sites. Fig. 16 shows three neurons about 40 μm from the tip of electrode #3 from IC-190, which was pulsed for 7 hours at 500 Hz and at 4 nC/phase. The neurons and neuropil appear to be normal.

DISCUSSION

In cat IC-176 the stability of the electrical threshold of the corticospinal neurons throughout 7 hours of stimulation at 4 nC/phase and at 50 Hz (Fig.2), is consistent with the absence of any histologically-detectable neural injury, 48 hours after the end of the stimulation (Fig 15). In cats IC-176 and 190, stimulation at 4 nC/phase and 500 Hz induced considerable elevation of the electrical threshold (Fig.3), but no histologically-detectable neuronal changes. This was also true in cat IC-192, in which a cluster of 11 microelectrodes was pulsed simultaneously for 7 hours, at 4 nC/phase and at 50 Hz. Thus, as we have reported previously (QPR's 3 and 7), moderate stimulation-induced depression of neuronal excitability (SIDNE) can occur in the absence of histologically-detectable tissue changes. This includes the absence of intracellular changes (mitochondrial and other organelles) when the evaluations were carried out by transmission electron microscopy.

Damage to cortical neurons incurred by high intensity electrical stimulation delivered by surface Pt disc electrodes (8 mm in diameter) is extensive and unmistakable by light microscopic evaluation (Agnew et al., 1993). In the tissue

beneath these electrodes, most of the neurons had become markedly shrunken, stellate, and hyperchromic and were surrounded by pericellular haloes. Not one instance of this type of neuronal injury was observed in this series utilizing multiple electrode microstimulations delivered continuously for 7 hrs/day for up to four days, using a wide range of stimulus frequencies (50 to 500 Hz) and a range of stimulus amplitude (4 or 8 nC/phase). Also encouraging is the lack of histologically-demonstrable neural injury in two animals whose cortex was pulsed with 11 closely-spaced microelectrodes. However, the studies indicate that the electrical excitability of neurons near the 11 closely-spaced electrodes is depressed. Previous studies (QPR's 3 and 7) indicate that the recovery of neuronal excitability is a slow process; one neuron required 28 days to recover to its prestimulus threshold.

Monitoring of the stability of the chronically implanted arrays by recording the action potential from individual corticospinal neurons has proved to be a helpful adjunct. The data indicate that "floating" subdural arrays may continue to move within the cortex. This is consistent with histologic findings of linear glial scars; e.g., Fig. 8, which indicate that the microelectrodes have been migrating slowly through the cortex. In the case of IC-175 (Figure 8), the physiologic data indicate that most of this migration took place in the first few months and thereafter the array became fairly stable.

These findings are of fundamental importance to the ultimate application of microstimulation to a visual prosthesis which would require prolonged pulsing(at least 12-hour/day) over the lifetime of the patient. Further work will be directed toward understanding the nature of SIDNE and to develop protocols to minimize this phenomenon during prolonged microstimulation with spatially dense arrays.

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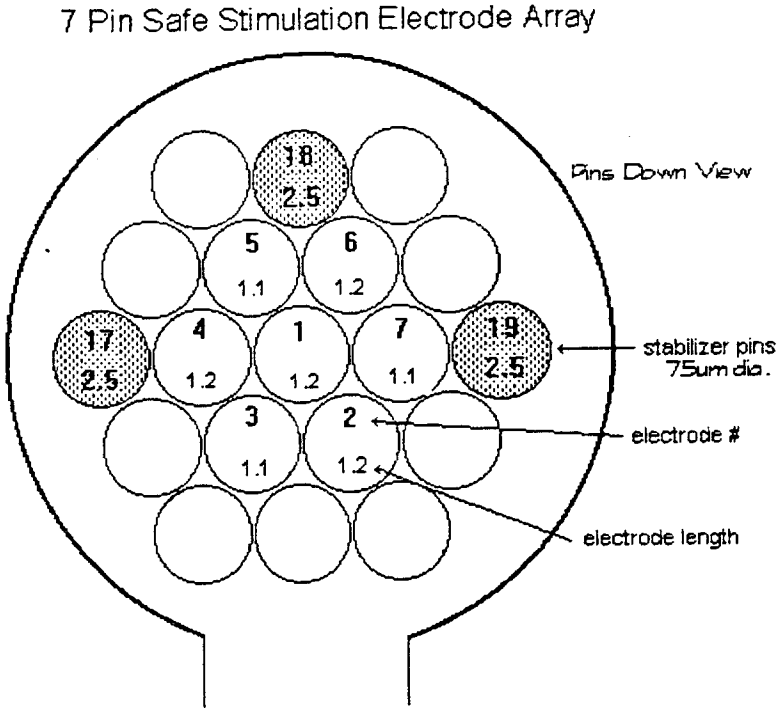

Fig. 1-A. Superior view of an intracortical array of 7 microelectrodes and 3 stabilizer pins (cross hatched). The length of the electrodes are indicated below the electrode number.

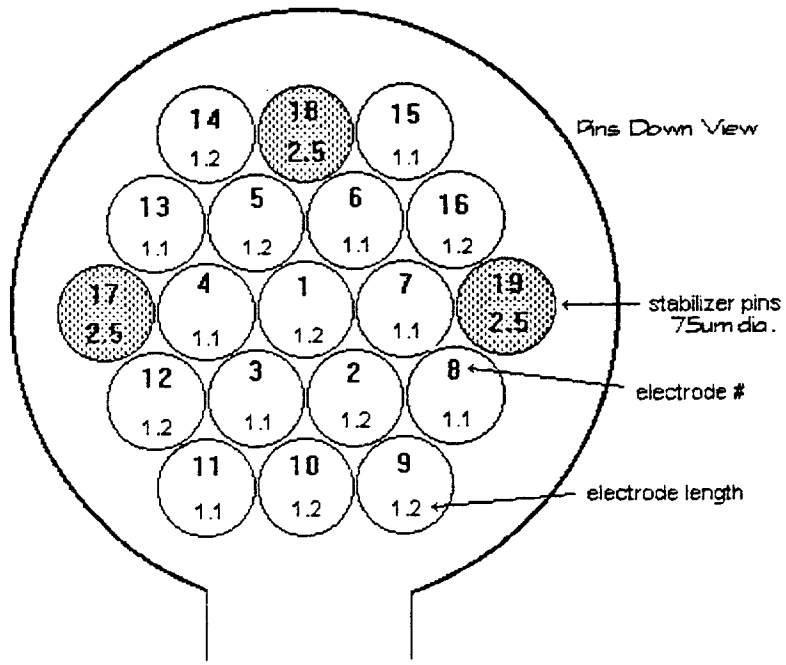

Fig. 1-B. A 16-microelectrode array with an additional 3 stabilizer pins (cross hatched). The lengths of the electrodes are indicated beneath the electrode number.

The effect of 7 hours of intracortical microstimulation on the threshold of pyramidal tract responses (Response latency < 2 ms)

Pulse rate = 50 Hz, Q= 4 nC/phase (26.5 μA)

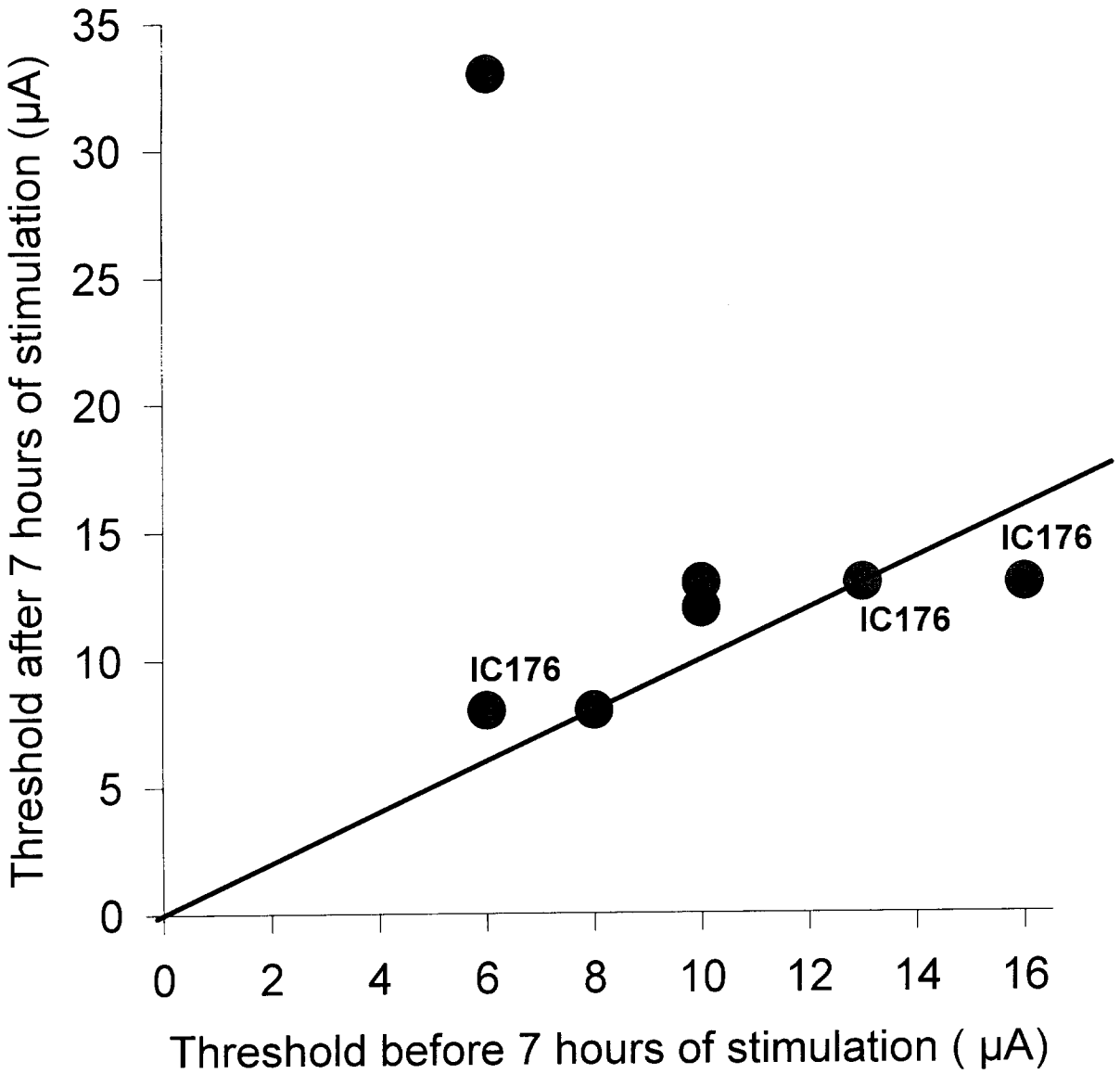

Figure 2

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The effect of 7 hours of intracortical microstimulation on the threshold of pyramidal tract responses (Response latency < 2 ms)

Pulse rate = 500 Hz Q= 4 nC, (26.5 µA)

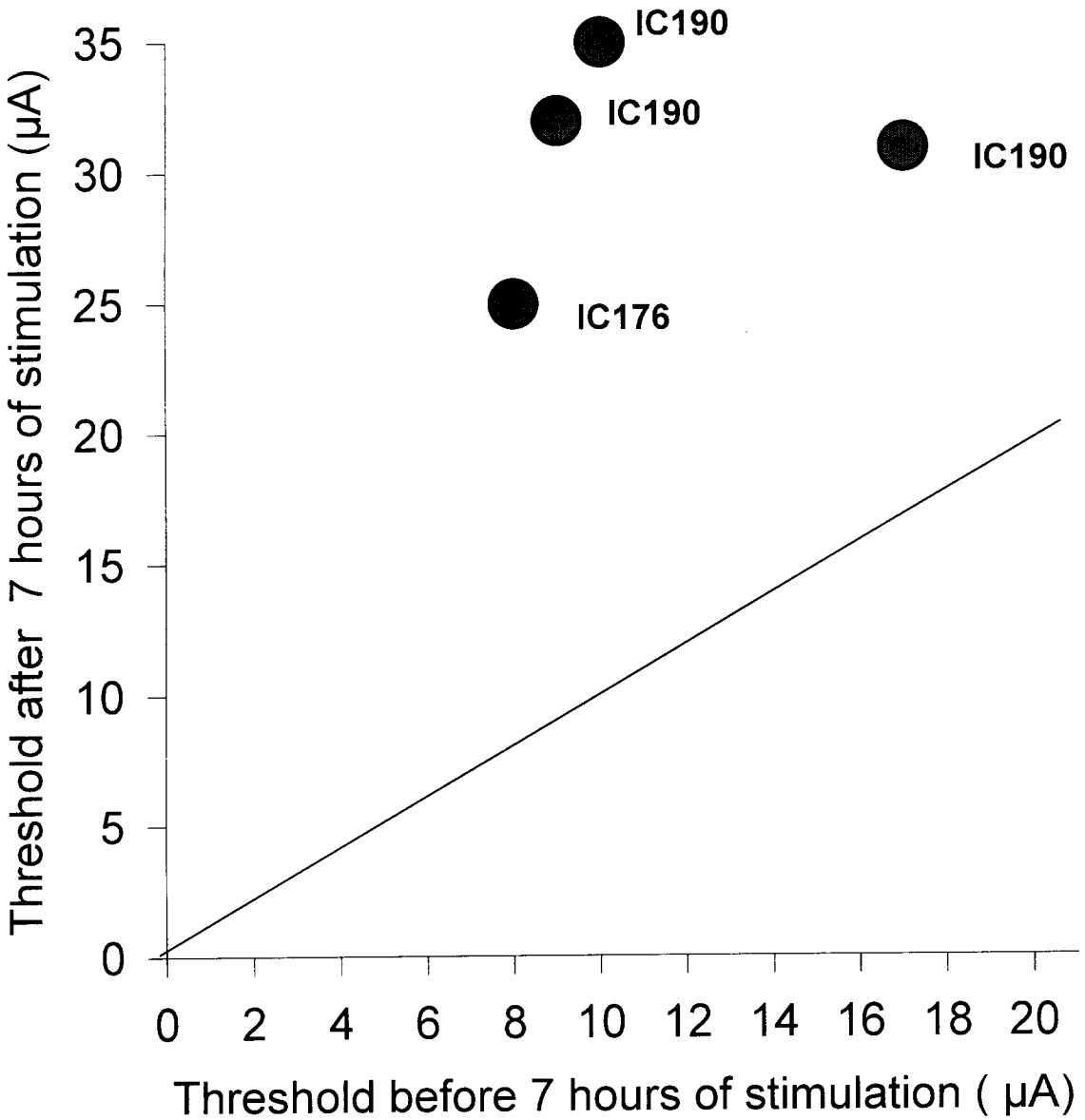

Figure 3

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IC192: 16-electrode array with
11 electrodes pulsed simultaneously

Pulse rate=50 Hz, Q= 4 nC (26.5 µA)

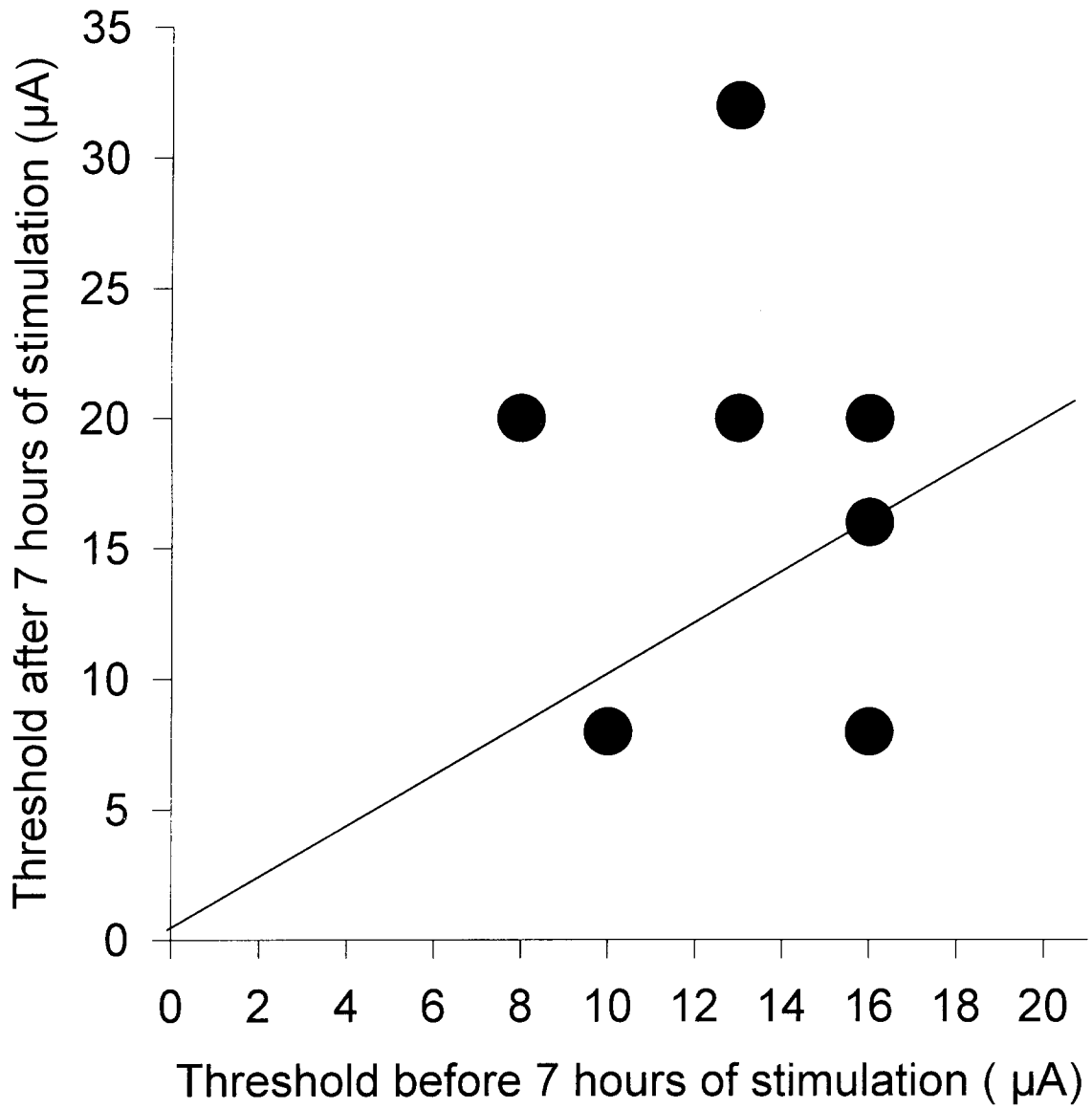

Figure 4

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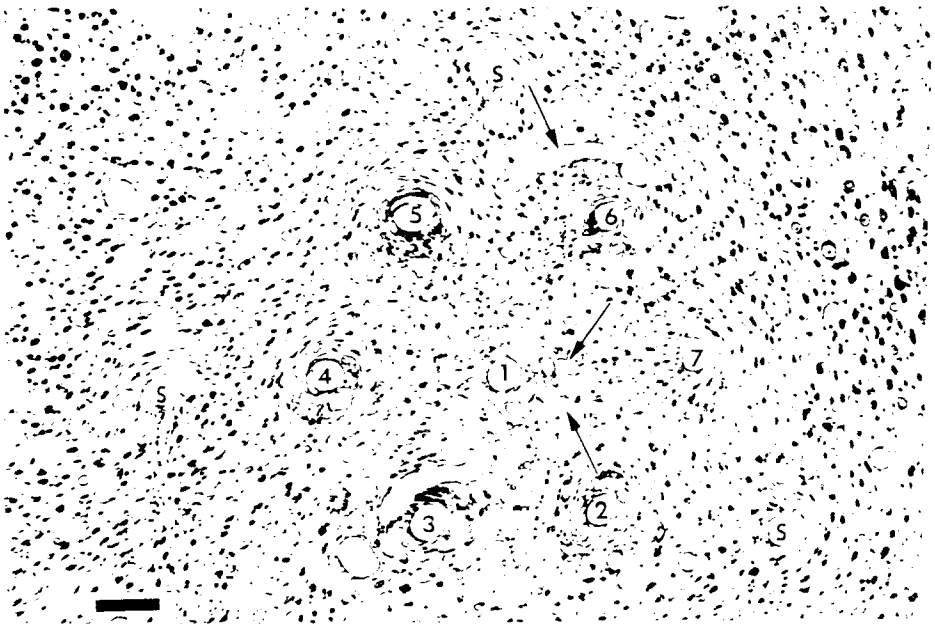

Fig. 5. Animal IC-175. Cross section through all 7 electrode tracks (1-7) and the tracks of three stabilizing pins (S) at a depth of 200 µm below the pia. Both vascular hyperplasia and hypertrophy are present (arrows). Blood vessels are closely opposed to the tracks of electrodes 5 and 6. Although not easily seen at this magnification, a few flattened neurons surround electrode no. 4. Nissl stain. Bar = 100 µm. Unless otherwise stated, all micrographs are from paraffin embedded blocks.

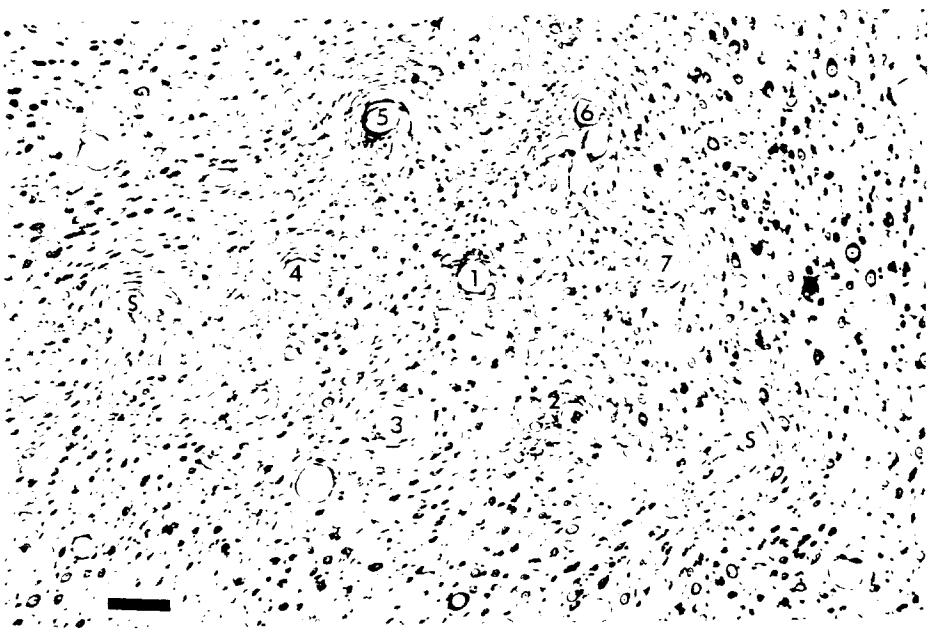

Fig. 6. Same animal as shown in Fig. 5 at a depth of 300 µm. Very little vascular hypertrophy and hyperplasia are seen at this level. Nissl stain. Bar = 100 µm.

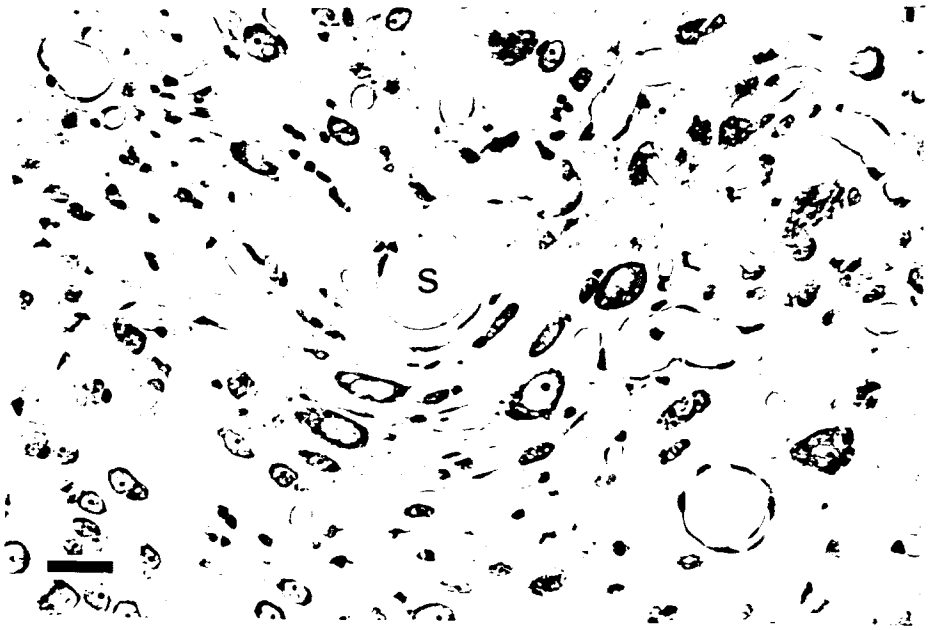

Fig. 7. IC-175. The track of the shaft of the anterior stabilizer pin at a depth of 300 µm, showing compression of the neurons and neuropil adjacent to the track. Nissl stain. Bar = 25 µm.

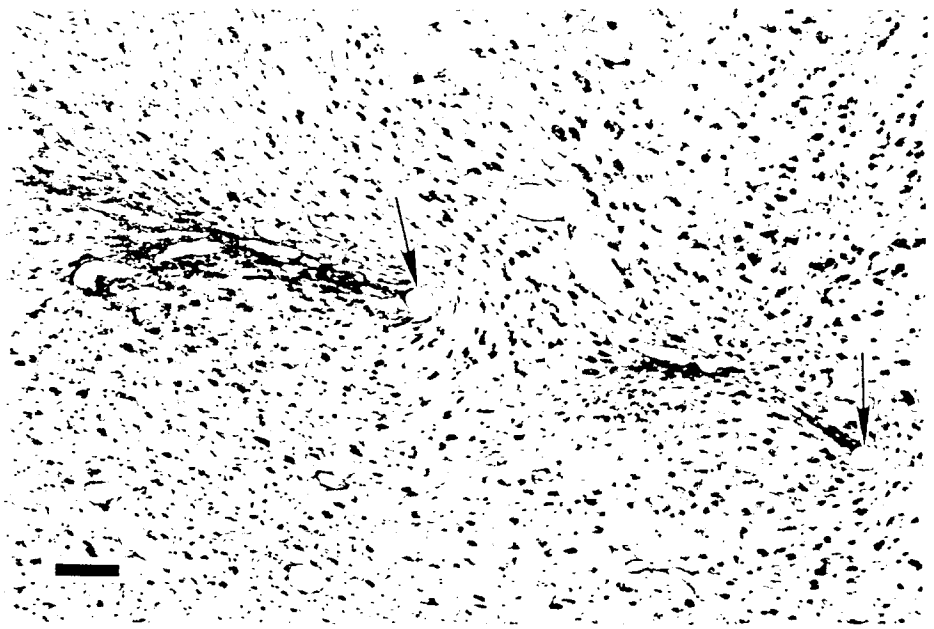

Fig. 8. IC-175. Tracks of 2 stabilizer pins (arrows) with linear glial scars near their tips. Note compressed neurons to the right of the central track. GFAP stain. Bar = 100 µm.

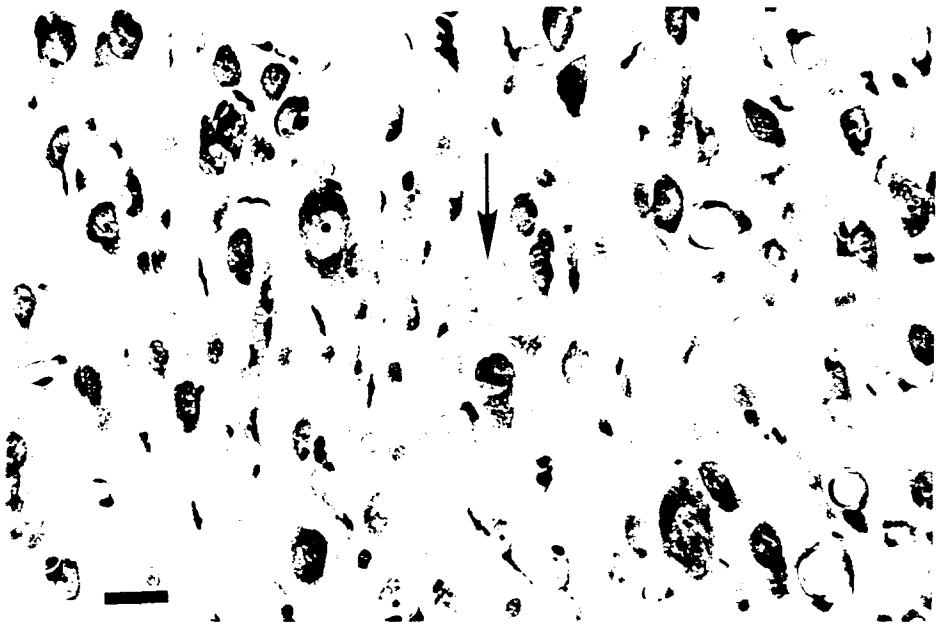

Fig. 9. IC-169. Tip site of pulsed electrode #6 (arrow), showing essentially normal-appearing neurons. Stimulus parameters: 4 nC/ph, 50 Hz, 7 hrs., sacrificed 4 days after end of stimulation. Nissl stain. Bar = 25 μm.

Fig. 10. IC-174. Site of the tip of unpulsed electrode #1 (arrow) showing a few glial cells and numerous normal-appearing neurons. Nissl stain. Bar = 25 μm.

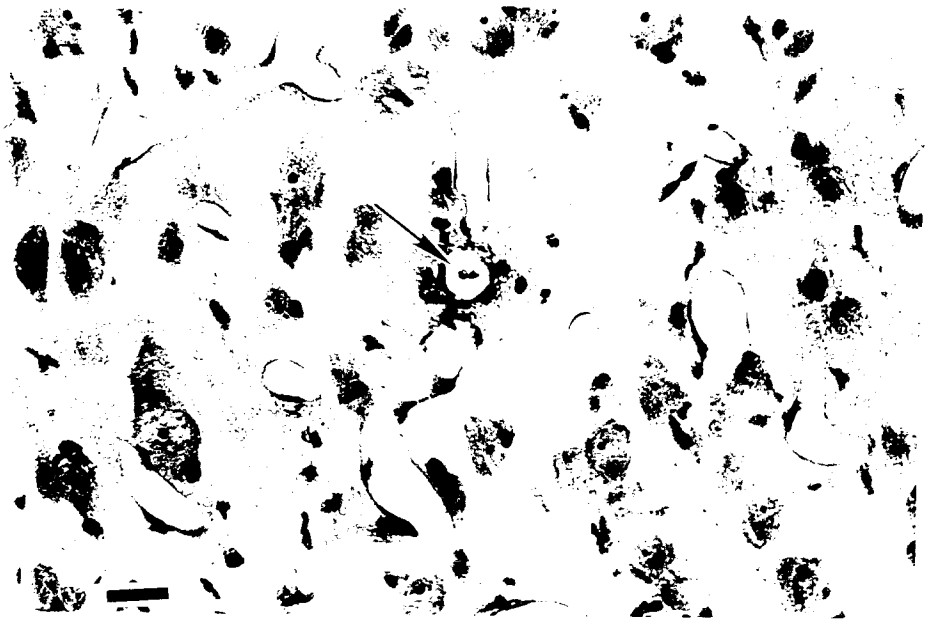

Fig. 11. IC-196. Tip site of pulsed electrode #1 (arrow). A few glial cells skirt the tip site. The surrounding tissue (including neurons) appears normal. Stimulus parameters: 7 hrs. × 3 days, 100 Hz, 8 nC/ph, cat sacrificed immediately after end of stimulation. H&E stain. Bar = 25 µm.

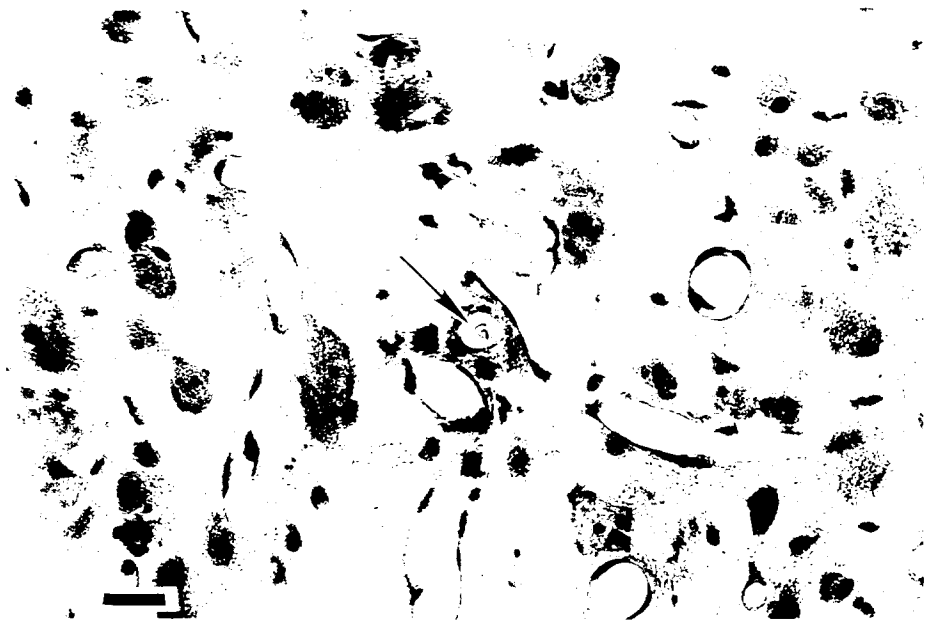

Fig. 12. IC-196. Tip site of pulsed electrode #16 (arrow) is surrounded by normal neuropil. Nissl stain. Bar = 25 µm.

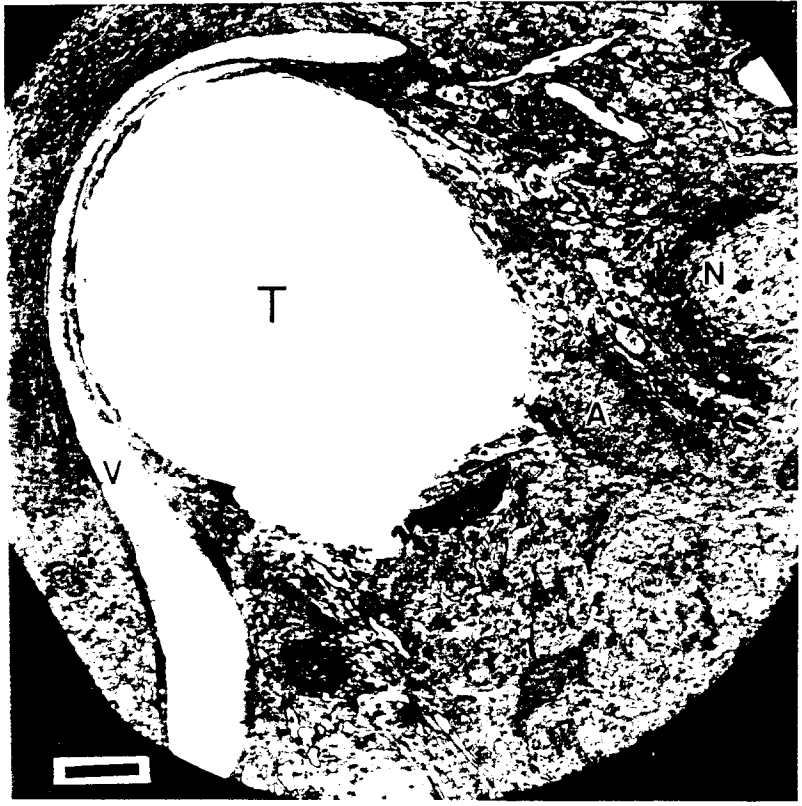

Fig. 13. IC-190. Electron micrograph of the track of unpulsed electrode #5 (T) about 75 µm above the tip . Note the normal-appearing neuron (N) approximately 15 µm lateral to the track. Note also the normal-appearing astrocyte (A). There was some post-mortem disruption of the sheath lining the track, when the electrode was removed. Note the "wraparound" vessel (V) at the left edge of the track. This may be the result of displacement of the vessel by the 12 µm diameter electrode tip. Stained with uranyl acetate and lead citrate. Bar = 7 µm.

Fig. 14. IC-190. One µm semi-thin plastic section showing a large, normal-appearing neuron (arrow) approximately 5 µm from the track (T) of pulsed electrode #6 and approximately 90 µm above the site of the tip. Other neurons in the field also appear normal. Stimulus parameters: 7 hrs., 500 Hz, 4 nC/ph, cat sacrificed immediately. Richardson's stain. Bar = 25 µm.

Fig. 15. IC-176. Electron micrograph through the track (T) of pulsed electrode #3, showing 2 normal-appearing neurons (N) approximately 15-20 µm from the track and approximately 12 µm above the site of the electrode tip. There is some edema in some of the surrounding neuropil but the tissue appears essentially normal. Stimulus parameters: 7 hrs. × 2 days, 50 Hz, 4 nC/ph, cat sacrificed immediately after end of stimulation. Stained with uranyl acetate and lead citrate. Bar = 5 µm.

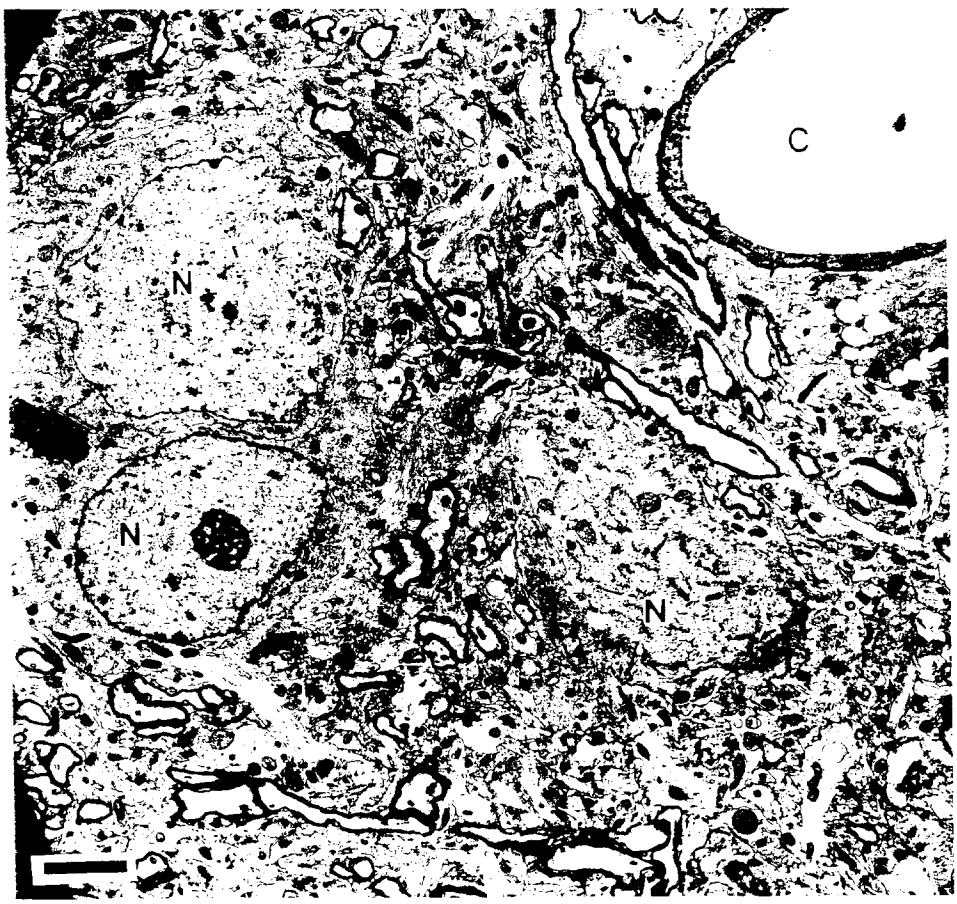

Fig. 16. Electron micrograph from IC-190, 40 µm above the tips site of pulsed electrode #3. The electrode track itself is not shown, There are normal-appearing neurons (N) approximately 40 µm lateral to the track, with normal cytoplasm and well-defined nuclei and nucleolus. The capillary (C) also appears normal. Stimulation parameters: 7 hrs., 500 Hz, 4 nC/ph, cat sacrificed immediately after end of stimulation. Stained with uranyl acetate and lead citrate. Bar = 3 µm.